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ORIGINAL ARTICLE

Spatial and seasonal variations in the biofunctional lipid substances (fucoxanthin and fucosterol) of the laboratory-grown edible Japanese seaweed (*Sargassum horneri* Turner) cultured in the open sea

Masaru Terasaki [a], Chikara Kawagoe [b], Atsushi Ito [b,1], Hiroko Kumon [b], Bhaskar Narayan [c,d,2], Masashi Hosokawa [c], Kazuo Miyashita [c,*]

[a] *Department of Health & Environmental Sciences, School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido 061-0293, Japan*
[b] *Algatech Kyowa, Kyowa Concrete Industry Co. Ltd., Hakodate, Hokkaido 040-0051, Japan*
[c] *Laboratory of Biofunctional Material Chemistry, Division of Marine Bioscience, Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan*
[d] *MMS, CSIR-CFTRI, Mysore 570 020, India*

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KEYWORDS

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Fucosterol;
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Abstract This work studied the effect of spatial and seasonal differences on the accumulation of functional lipid components in *Sargassum horneri* (Turner), an edible Japanese seaweed popularly called *Akamoku*. *S. horneri* obtained from Samenoura bay area of Japan was laboratory cultured to evaluate the effect of temperature on the accumulation of total lipids (TL), fucoxanthin (Fx) and fucosterol (Fs) by the alga. The laboratory cultured 3 month old *S. horneri* were cultured in the open sea in two different geographical locations off Usujiri and Matsushima to evaluate the monthly variations, over a year, in their TL, Fx and Fs contents. *S. horneri* grown off the Usujiri area accumulated the maximum TL close to 193 mg g^{-1} dry weight during the coldest part of the year. Fx and Fs contributed 5.6% and 16.2% of the TL in *S. horneri* harvested off Usujiri in February. Further, in spite of being the same species and parent stock, *S. horneri* grown off the

Abbreviations: dwt, dry weight; Fs, fucosterol; Fx, fucoxanthin; TL, total lipids

* Corresponding author. Tel./fax: +81 138 40 8804.
E-mail address: kmiya@fish.hokudai.ac.jp (K. Miyashita).
[1] Present address: National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Onomichi, Hiroshima 722-0061, Japan.
[2] JSPS Invitation Fellow at Hokkaido University at the time of this work.

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Matsushima area accumulated less TL, Fx and Fs as compared to their Usujiri counterparts. Our study clearly indicates the role of temperature and light apart from nutritional profile and depth of waters where the seaweed was grown on the accumulation of functional lipid components in *S. horneri*.

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1. Introduction

Marine foods have long been regarded as rich sources of healthy lipids including n-3 and n-6 poly-unsaturated fatty acids (PUFA). Till a few decades ago marine lipids were only synonymous with eicosapentenoic acid and docosahexenoic acid. In the recent times, several researchers have conclusively shown that other lipid substances like carotenoids and sterols are also equally beneficial for human health (Mori et al., 2004; Bhaskar et al., 2006; Hosokawa et al., 2006; Miyashita et al., 2011; Wang et al., 2014). Seaweeds or macro-algae are known to be the major sources of PUFA and carotenoids in fish/shellfish as the latter cannot synthesize these compounds (Nomura et al., 2013). Among the marine carotenoids, fucoxanthin (Fx) – a xanthophyll – is an abundant marine carotenoid that is responsible for the coloration of brown seaweeds. Several researchers have conclusively reported the antioxidative (Sachindra et al., 2007; Airanthi et al., 2011), anti-cancerous (Miyashita et al., 2011), anti-diabetic (Maeda et al., 2005; Nishikawa et al., 2012) and anti-obesity effects including the unique molecular mechanism responsible for the anti-obesity effects (Maeda et al., 2005; Miyashita et al., 2011) of Fx. Like Fx, fucosterol (Fs) is a characteristic sterol mainly found in brown seaweeds (Sanchez-Machado et al., 2004). It is also known to have several health benefits including hypercholesterolemic activity by lowering the absorption of cholesterol (Ikeda et al., 1988), anticancerous properties (Sheu et al., 1999; Hosokawa et al., 2010) and antioxidative characteristics (Lee et al., 2004). It is very interesting to note that both these unique molecules are available in various brown seaweed species as reported by different researchers (Terasaki et al., 2009; Le Lann et al., 2012; Terasaki et al., 2012; Nomura et al., 2013). Owing to these beneficial compounds, brown seaweeds could well be prospective sources of healthful lipids for the benefit of mankind.

In the recent past, some researchers including our group, have reported variations in fatty acids, total phenolics, Fx and/or Fs in several species of edible brown seaweeds harvested from the different marine environs around the Japanese coast (Mori et al., 2004; Terasaki et al., 2009; Nomura et al., 2013), New Zealand coast (Wang et al., 2014), off South Pacific ocean from the Fiji Island (Le Lann et al., 2012), from Mauritius coast (Ramah et al., 2014) and a few coastal areas of the USA (Rosenburg and Ramus, 1982). The spatial and seasonal variability in the contents of the lipid substances as a growth strategy has also been proposed by many of these groups. Recently our group reported the seasonal variations in Fx and Fs in different brown seaweed species as a function of season (Terasaki et al., 2009; Terasaki et al., 2012; Nomura et al., 2013) and location (Nomura et al., 2013). *Sargassum horneri* an edible brown seaweed, popularly known as *Akamoku* in Japan, was found to be a major source of Fx and Fs and could be easily propagated through initial culture in the laboratory and later in the open sea. Our group has earlier reported that *S. horneri* harvested from colder waters (Terasaki et al., 2009; Terasaki et al., 2012) and colder periods (Nomura et al., 2013) contained higher TL, Fx and Fs contents; and, attributed it possibly to the temperature and light conditions. Against this backdrop, the objective of the present study was to evaluate the effect of spatial and seasonal variability on the contents of Fx and Fs in laboratory grown but open sea cultured *S. horneri* in two different locations. Further, our aim also was to use this strategy to develop sustainable resources of biofunctional materials that can be used in the development of innovative food/food ingredients.

2. Materials and methods

2.1. Materials

Mature thalli of *S. horneri* for culturing in the laboratory were obtained at a depth of 3 meter from the sea off Samenoura Bay area (38°23′N 141°31′E) of Miyagi prefecture during May 2007. Young thalli from the laboratory cultured *S. horneri* were used for studies involving open sea culture.

Standard fucoxanthin (Fx; >99% purity as established by HPLC) for use in this study was purified from *Undaria pinnatifida* lipids as described previously (Maeda et al., 2005). Standard fucosterol (Fs; >99% purity) was from Extra-synthase (Munich, Germany). Only HPLC grade solvents were employed for RP-HPLC analysis of Fx and Fs. All other solvents and chemicals used in the study were of analytical grade, unless otherwise mentioned.

2.2. Methods

2.2.1. Laboratory culturing of S. horneri

The fertilized eggs adhering to the female receptacle of mature *S. horneri*, as mentioned above, were confirmed by microscopy. Several female receptacles were cut, placed into glass bottles filled with sterile seawater and incubated for 2 days at 4 °C for allowing the receptacles to release the eggs. The fertilized eggs so released were washed on the gauze with sterile seawater, transferred to 50 mL vial and the washing procedure was repeated five more times before finally storing the eggs in sterile seawater in a 50 mL vial. The fertilized eggs developed into embryos on the 3rd day. The embryos were then cultured in a 100 mm glass dish, in batches, [with a photoperiod of light/dark cycle of 12 h; irradiance of 40 $\mu E/m^2/s$] at 15 °C till they reached a length of ~3 mm. The immature thalli (~3 mm length) were further cultured with nutrient (F-12) at 20 °C under light/dark cycle of 12 h [irradiance of 40 $\mu E/m^2/s$]. The growing thalli were brushed and culled every week along with replacement of growth medium for 3 months, by

which time the thalli reached a mean length of 5.0 cm and mean weight of 2.0 g. These harvested young thalli were then taken for culturing in the open sea. However, for comparing it to the open sea culture experiments, the laboratory growth experiments were continued up to 12 months, in two different temperature regimes (15 or 20 °C) spread over a particular period, by following the brushing and culling procedure above. The different conditions included – (i) constant temperature of 20 °C for 3 months, (ii) constant temperature of 20 °C for 12 months, and (iii) constant temperate of 20 °C for 9 months followed by reducing it to a constant temperature of 15 °C for the next 3 months).

2.2.2. Open sea culture of laboratory grown seaweed

Young thalli of *S. horneri* (mean length 5.0 cm, mean weight 2.0 g) after 3 month culture (at 20 °C) (Fig. 1A) were planted in two different areas viz., off the coast of Usujiri (41°94′N 140°94′E) and off Matsushima area (38°22′N 141°5′E). In case of Usujiri area, the young thalli were twisted into ropes at a distance of 50 cm from each other and these ropes were suspended at 3 to 15 m of depth in the middle of September 2007; and, the adult algae were collected by the end of February 2008 when they had reached an average length of 1.0 m (Fig. 1B). Likewise, in case of Matsushima sea area, the young thalli were twisted into ropes at a distance interval of 30 cm and were suspended in the sea horizontally at a depth of 1 m using rope frame at the end of August 2007. Samples were drawn every month to assess the length and weight of the thalli set into ropes. Data logger was employed to profile the sea temperature and duration of sunshine during the course of the study.

2.2.3. Extraction of total lipids from S. horneri

All the extractions were carried out under dim yellow light and air in the extraction vessel was replaced with nitrogen, unless otherwise mentioned, to prevent any possible degradation of carotenoids or lipids. Total lipids (TL) from seaweeds were obtained by overnight extraction of seaweeds (~10 g; wet weight) with methanol (1:10 w/v). The filtrate was kept in screw-capped bottles (250 ml) after flushing the headspace with nitrogen till further analysis. The residue was again overnight extracted with methanol as mentioned above to collect the filtrate again. Both the filtrates were pooled and the solvent removed under vacuum at 30 ± 1 °C using a rotary flash evaporator (Eyela N1000; Tokyo Rikakikai Ltd., Tokyo, Japan) to obtain a viscous green residue. The last traces of solvent remaining in the extract were removed under high vacuum. The viscous green residue thus obtained was referred to as TL. TL was weighed, re-dissolved in HPLC grade methanol, and stored at −35 °C until further analysis.

2.2.4. Analysis of fucoxanthin and fucosterol from TL extract

An aliquot of TL was dissolved in acetone, filtered with a 0.22 μm of membrane filter (PTFE Acrodisc; Wako, Osaka, Japan) and subjected to HPLC analysis. The fucoxanthin and fucosterol standards along with seaweed TL extracts were subjected to RP-HPLC on an HPLC system (Elite LaChrom L-2130; M/s Hitachi High Technologies, Tokyo, Japan) equipped with binary pump, an autosampler (Elite LaChrom L-2200), a photodiode array (PDA) detector (Elite LaChrom L-2455 photodiode array detector) and equipped with the manufacturer provided analytical software (EZChrom Elite).

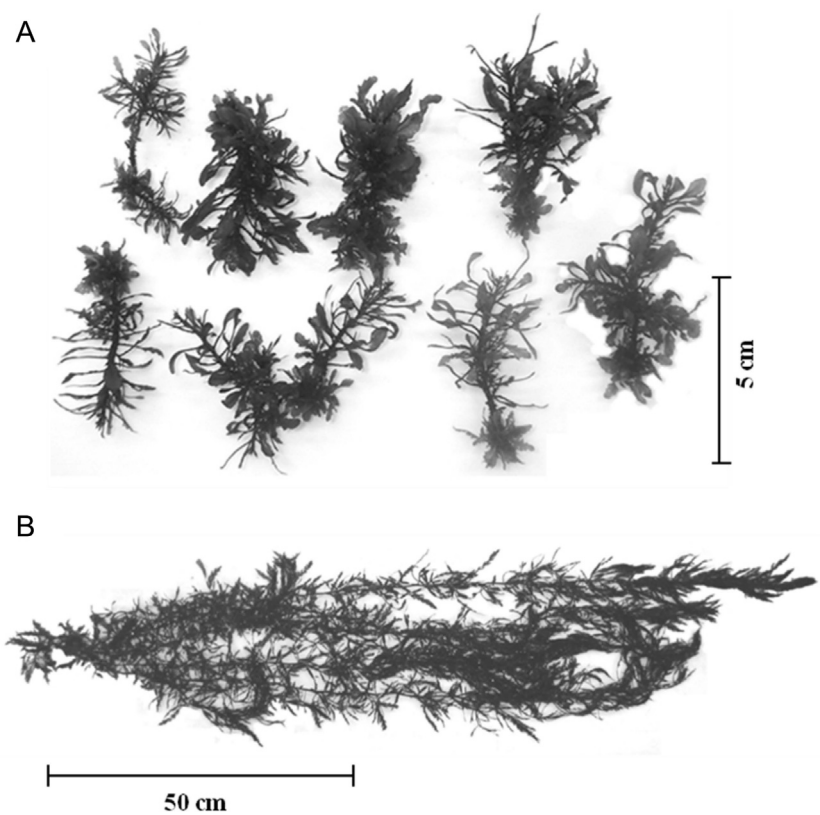

Figure 1 Photograph showing (A) young thalli of *S. horneri* grown in the laboratory ready for placing into open sea for culture; and (B) adult algae after culturing in open sea for ~6 months.

Table 1 Total lipids (TL; mg g^{-1}), fucoxanthin (Fx; mg g^{-1}) and fucosterol (Fs; mg g^{-1}) contents (all on dry weight basis) in *Sargassum horneri* under different culture conditions in the laboratory.

Sample	*n*	TL	Fx	Fx_{TL} #	Fs	Fs_{TL} #
3 M (20 °C)	3	55.8 ± 10.6	3.4 ± 0.4	6.1	14.5 ± 1.1	26.0
12 M (20 °C)	3	51.7 ± 7.4	3.9 ± 1.2	7.5	12.3 ± 1.3	23.8
9 M (20 °C) →3 M (15 °C)	4	116.8 ± 4.8	12.0 ± 0.9	10.3	32.3 ± 2.8	27.7

Whole body of seaweed was taken for analysis; all the values are mean ± SD except for values in column marked with #; M: months; *n*: number of samples taken for analysis.
Contents of Fx and Fs expressed as percentage of TL.

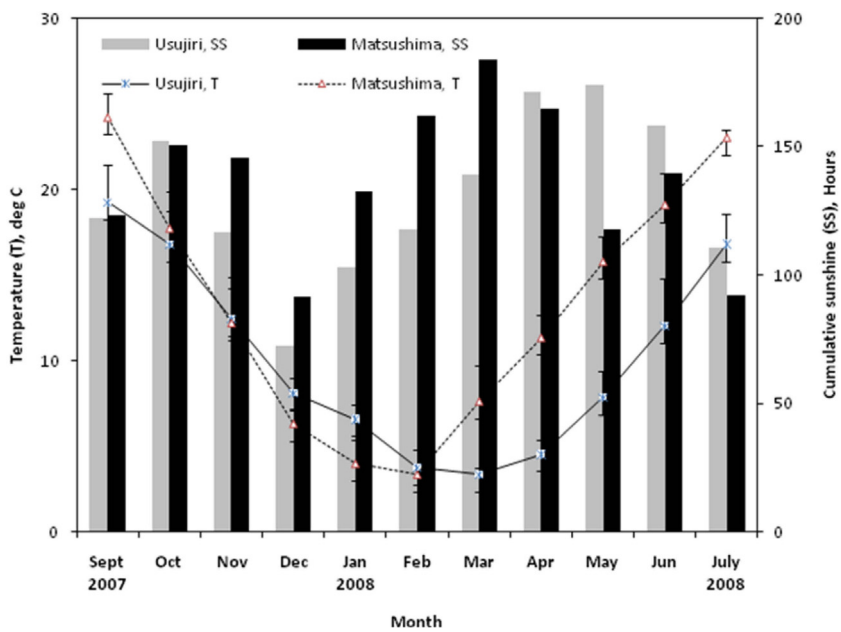

Figure 2 Average monthly water temperature (°C) and duration of sunshine (hours) off Usujiri and Matsushima sea areas obtained from the data logger during the course of the study.

All the HPLC analysis were carried out at 25 °C using a RP-HPLC column (TSK-gel ODS 80-Ts, 250 × 4.6 mm i.d., 5 μm particle size; Tosoh, Tokyo, Japan) protected with a ODS guard column (15 × 3.2 mm; Tosoh, Tokyo, Japan). The mobile phase was methanol-acetonitrile (7:3, v/v) at a flow rate of 1.0 mL/min. The detection wavelengths were set at 450 and 210 nm for detecting Fx and Fs, respectively. Standard curves prepared using authentic standards were used for quantification of Fx and Fs contents in the algal samples. Fx and Fs contents were expressed as mg g^{-1} of TL as well as mg g^{-1} of respective algal samples.

3. Results

3.1. Effect of temperature on TL, Fx and Fs contents of laboratory grown ***S. horneri***

TL content (mg g^{-1}) of batch cultured varied from close to 56 at the end of the 3rd month to about 52 after 12 months when grown at a constant temperature of 20 °C (Table 1). However, the content of TL (mg g^{-1}) went up by almost 2.1 fold when the culture temperature was reduced to 15 °C for 3 months in the period of 12 months. Likewise, Fx content almost quadrupled due to the reduction in temperature. Similar observations were also on display in the case of Fs as well whose content increased by 2.3-folds as a response to temperature reduction (Table 1). It should be noted that in this batch culture experiment all the factors (i.e., quantity and duration of light, medium composition and duration of culture) were kept constant except for change in culture temperature depending on the batch, as previously mentioned. Further, the thalli were brushed and culled in all the batches regularly at 3 month interval which mean that even those morphometric factors were kept in check. This clearly points to the fact that temperature plays a major role in *S. horneri* accumulating TL, Fx or Fs thereby elucidating the influence of temperature on the accumulation of functional lipid components in this seaweed.

3.2. Effect of spatial and seasonal variation on growth profile and functional lipid components of open sea cultured S. horneri

The average monthly temperature (°C) and monthly cumulative sunshine duration (hours) at the culture site are presented in Fig. 2. Further, the length and weight of thalli placed for culture at different periods of sampling along with corresponding average temperatures and sunshine hours are presented in Table 2; while, Fig. 3 presents the variations in TL content (mg g^{-1}) over the culture period at both Usujiri and Matsushima areas.

Table 2 Growth profile of *Sargassum horneri* in different waters vis-à-vis water temperature (WT; °C) and duration of sunshine (DS; hours) during culture in open sea.

Sampling period	WT, °C		DS, h	*n*	Length, cm	Weight, g*
	day@	interval#	interval[a]			
Usujiri						
Oct 24, 2007	14.7	18.2	174.2	6	18.0 ± 4.2	5.7 ± 2.1
Nov 26, 2007	11.3	13.4	122.9	6	14.8 ± 6.5	5.0 ± 5.1
Dec 20,2007	7.0	8.8	72.0	5	45.8 ± 20.6	27.8 ± 12.5
Jan 23, 2008	6.9	7.1	103.8	4	101.8 ± 29.8	101.1 ± 49.9
Feb 29, 2008	2.8	4.3	139.7	5	101.4 ± 42.0	131.9 ± 83.3
March 21, 2008	3.5	3.2	83.8	6	93.6 ± 17.6	143.7 ± 138.6
Apr 17, 2008	5.0	3.9	141.5	5	158.6 ± 39.3	287.7 ± 311.0
May 23, 3008	8.9	6.4	238.4	6	207.6 ± 76.1	646.2 ± 623.3
Jun 19, 2008	13.6	10.5	120.6	6	215.8 ± 72.4	628.8 ± 183.5
Jul 24, 2008	17.4	15.5	144.8	5	325.0 ± 116.0	1213.6 ± 774.2
Matsushima						
Oct 22, 2007	15.3	22.1	224.9	5	19.7 ± 16.2	8.8 ± 11.4
Nov 20, 2007	10.5	14.7	140.4	7	59.6 ± 31.3	72.8 ± 66.0
Dec 17, 2007	6.0	7.8	107.0	4	58.9 ± 8.8	46.9 ± 25.5
Jan 21, 2008	2.8	5.2	124.1	7	81.0 ± 30.3	232.5 ± 461.5
Feb 15, 2008	3.3	2.8	122.2	5	92.4 ± 28.5	79.4 ± 53.2
March 3, 2008	5.0	3.9	101.6	2	152.0 ± 22.0	514.3 ± 132.5
Apr 23, 2008	12.8	9.2	276.7	1	113.0	417.6
May 12, 2008	13.2	14.4	109.7	1	69.0	79.8
June 23, 2008	20.1	17.4	184.0	1	130.0	262.8

n: number of samples used for growth determination, values expressed as Mean ± SD.
@ Water temperature on the day of sampling.
# Average temperature during the interval between two sampling days.
[a] Total sunshine (cumulative) hours during the interval between two sampling days.
* Wet weight basis.

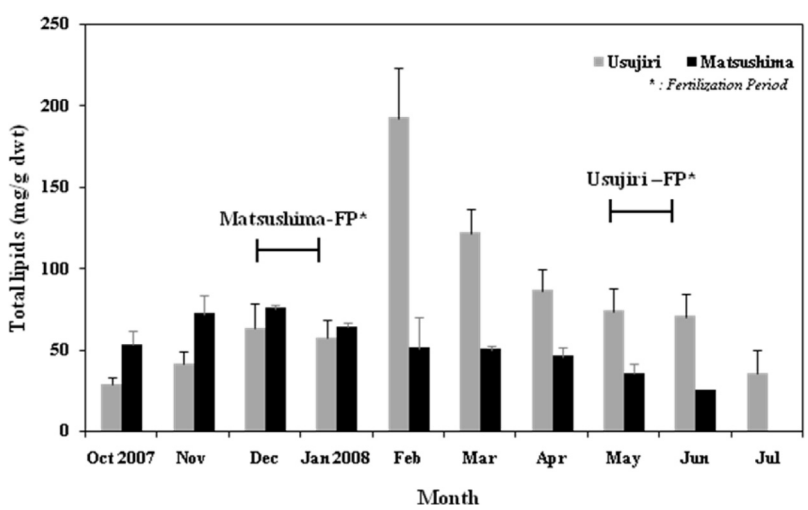

Figure 3 Monthly variations in total lipids (TL; mg g^{-1} dry weight) of *S. horneri* cultured in open sea off Usujiri and Matsushima sea areas. (*FP: fertilization period of the algae in the selected sites).

In addition, as mentioned elsewhere, the only variation in either of the area was the depth to which the rope was lowered (1 m in Matsushima and 3–15 m in case of Usujiri). It becomes clear from Figs. 2 and 3 that the depth at which the thalli were placed had an influence on the growth, accumulation of biofunctional components. For instance, off Usujiri the thalli accumulated more TL as compared to Matsushima sample during the colder period of the year (Fig. 3), although variations in monthly average temperature and cumulative sunshine hours were not considerably different. This is indicative of the

Table 3A Fucoxanthin (Fx; mg g^{-1}) and fucosterol (Fs; mg g^{-1}) contents (all on dry weight basis) in laboratory cultured *Sargassum horneri* grown in waters off Usujiri sea area.

Sampling Day	*n*	Fx	Fx_{TL} #	Fs	Fs_{TL} #
Oct 24, 2007	3	1.0 ± 0.3	3.5	4.1 ± 0.7	14.2
Nov 26, 2007	3	2.2 ± 1.0	5.3	7.4 ± 2.6	17.9
Dec 20, 2007	3	3.7 ± 0.3	5.8	14.8 ± 3.2	23.3
Jan 23, 2008	3	3.4 ± 0.5	5.9	10.8 ± 1.4	18.8
Feb 29, 2008	3	10.8 ± 2.6	5.6	31.2 ± 7.0	16.2
Mar 21, 2008	3	4.6 ± 1.8	3.8	14.9 ± 4.3	12.2
Apr 17, 2008	3	4.0 ± 0.4	4.6	12.7 ± 1.2	14.7
May 23, 2008	1	4.0	5.4	14.1	19.1
Jun 19, 2008	3	3.8 ± 1.3	5.4	13.9 ± 2.1	19.7
Jul 24, 2008	3	1.1 ± 0.3	3.1	14.3 ± 5.6	40.6

Whole body of seaweed was taken for analysis; all the values are mean ± SD except for values in column marked with #; M: months; n: number of samples taken for analysis.
Contents of Fx and Fs expressed as percentage of TL.

fact that temperature in combination with the depth at which the seaweeds were grown had an influence on the accumulation of TL or other lipid substances.

Further, a similar trend was also observed in case of Fx and Fs contents (mg g^{-1}) of *S. horneri* harvested from the Usujiri site (Table 3A) wherein the colder periods saw higher contents of Fx and Fs. However, in comparison, the Matsushima harvested samples had less amount of Fx and Fs (Table 3B) although the parent stock placed for culture in both the areas was same. This clearly indicates the effect of depth on the accumulation of biofunctional lipids in *S. horneri*. It is a known fact that as the depth increases the temperature drops in the ocean as compared to the surface and probably this would have helped *S. horneri* in Usujiri area, placed at 3–15 m depth, to accumulate more TL, Fx and Fs as compared to their Matsushima counterparts placed at 1 m depth. Another factor could have been the maturation period for *S. horneri* in the respective areas (Fig. 3) wherein the thalli in Matsushima area show fertilization closer to the colder months while those in Usujiri show after the colder months.

Table 3B Fucoxanthin (Fx; mg g^{-1}) and fucosterol (Fs; mg g^{-1}) contents (all on dry weight basis) in laboratory cultured *Sargassum horneri* grown in waters off Matsushima sea area.

Sampling Day	*n*	Fx	Fx_{TL} #	Fs	Fs_{TL} #
Oct 22, 2007	3	3.1 ± 0.6	5.8	10.1 ± 1.8	19.0
Nov 20, 2007	3	3.3 ± 1.3	4.6	11.7 ± 4.3	16.2
Dec 17, 2007	3	3.0 ± 0.9	4.0	11.1 ± 1.5	14.7
Jan 21, 2008	3	2.5 ± 0.3	3.9	7.6 ± 1.5	11.9
Feb 15, 2008	3	1.5 ± 0.7	2.9	6.0 ± 2.4	11.7
Mar 3, 2008	2	1.9 ± 0.7	3.8	5.0 ± 1.2	10.0
Apr 23, 2008	2	0.8 ± 0.4	1.7	8.5 ± 0.9	18.5
May 12, 2008	2	1.0 ± 1.4	2.8	7.0 ± 0.9	19.8
Jun 23, 2008	1	1.0	3.9	3.9	15.3

Whole body of seaweed was taken for analysis; all the values are mean ± SD except for values in column marked with #; M: months; n: number of samples taken for analysis.
Contents of Fx and Fs expressed as percentage of TL.

4. Discussion

It should be noted that fertilization period of the seaweeds vary depending on the tidal pools, phyotosynthetic periods, spermatia concentration, etc. in a given area (Engel and Destombe, 2002) which explains the time frame at which the fertilization happens in the sampling areas. Records indicate that duration of sunshine, PAR, and seawater temperature were the lowest for the areas, from which samples were collected, during February (Miyashita and Takagi, 1987). The present report also corroborates with this and other earlier reports (Terasaki et al., 2009; Nomura et al., 2013) for these areas. Similarly, contents of Fx, chl a, and chl c in the sporophyte of *U. pinnatifida* have been reported to be higher in winter and spring than in summer in both juvenile and adult sporophytes (Campbell et al., 1999). Our present study also demonstrated that TL, Fx and Fs contents of five brown seaweeds – from Hakodate area including *S. horneri* – were highest during the period in winter and spring (Terasaki et al., 2009). Further, our results from the laboratory cultured *S. horneri* in this study corroborate the impact of temperature on the accumulation of these functional lipid components.

In addition, it has been reported that low light during the winter period leads to an increased production of Fx in brown algae through the xanthophyll-cycle pathway that involves the formation of fucoxanthin from zeaxanthin via antheraxanthin, violaxanthin (Vx), and diadinoxanthin (Ddx) (Eonseon et al., 2003); and, as a general rule, algae possessing the Vx cycle usually possess Ddx. Fx makes a complex with chl-protein and plays an important role in light harvesting and photoprotection for effective light utilization and up-regulation of photosynthesis (Eonseon et al., 2003; Mouget et al., 2004). This strong correlation between the photosynthetic activity of brown seaweed life cycle with Fx coupled with temperature and light could possibly be the reasons for seasonal variations observed in the present study. It may be speculated that temperature coupled with light could also be the reason for increase in Fs content during colder periods, which possibly could be the response from the seaweeds to keep the cell membrane integrity at times of lower temperature and impaired photosynthetic periods.

Some researchers have attributed differences in TL or Fx contents of a species to genetic variations compared to samples of the same species from a different area (Hu et al., 2011; Nomura et al., 2013). However, in our study, though the samples were sourced from the area closer to Matsushima, the content accumulation was higher in Usujiri as compared to the same place from where they were harvested. In view of this, the role of nutritional profile waters in the respective areas in the observed difference in the functional lipid components cannot be ruled out. Further, as mentioned in the results the observed fertilization and maturation period for *S. horneri* in Matsushima was just before the colder months (i.e., closer to February 2008) whereas the Usujiri counterparts matured and fertilized much before (i.e., towars the end of May 2008) entering the colder phase (Fig. 3). This difference in maturation could also have played an important role in the observed differences in functional lipid components. In essence, our study indicates that several factors like maturation period, nutritional profile of water and depth also play an important role in association with the major factors (i.e., temperature

and light) which in turn result in differential accumulation of functional lipid components in *S. horneri*, though same genotype was cultured in different areas.

5. Conclusions

Our study clearly points to the fact that (a) effects of temperature, light and depth on TL, Fx and Fst in closely monitored conditions is required to be evaluated to develop protocols for obtaining thalli that can produce higher amount of biofunctional components, and (b) development of such protocol would afford younglings that can be cultured in feasible areas for sustainable production of biofunctional materials for use in food/pharma/feed applications. In turn it will also add to the economy of the fishermen community and co-operatives. These are the few areas wherein further research needs to be focused to develop sustainable resources that provide healthful components for benefits of humans.

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